Obligated Schooling of Threadfin Shad During Simulated Transportation: Effects on Stress, Injury, and Survival.

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Summary

Operations at Reclamation's Tracy Fish Collection Facility (TFCF; Central Valley Project) and John E. Skinner Delta Fish Protection Facility (State Water Project) function to salvage and maintain fish prior to encountering water pumping facilities, after which fish are truck transported for release outside of the immediate influence of the pumping facilities. Such operations result in the annual truck transportation of millions threadfin shad (*Dorosoma petenense*), the most abundant species of fish salvaged at the TFCF over the last decade (USBR 2009). To comply with the Biological Opinion, Reclamation is required to conduct research with the intention to initiate facility changes that improve survival of fish throughout collection, holding, transport, and release. To date, research has been aimed at minimizing stressors associated with, and improving survival during, fish loading and transport by investigating densities necessary to meet appropriate water quality conditions during transport (Sutphin and Wu 2009; Sutphin and Myrick, unpublished data). However, threadfin shad generally congregate in large schools (Moyle 2002) and fish salvage data indicates dense schools of shad can rapidly inundate both fish facilities making truck transportation of fish at stressful densities a necessity (Foss 2001, 2002). It is therefore necessary to investigate methods to minimize stress, external tissue damage, and survival during truck transportation of high densities of threadfin shad.

Fish schools, defined as a synchronized group with neighboring individuals orienting in a parallel position (Pitcher 1983, Iguchi *et al.* 2002), enhance forage potential and improve protection against predators (Moyle and Cech 2000; Tien *et al.* 2004), as well as reduce energetic output by improving hydrodynamic efficiency (Moyle and Cech 2000). Interestingly, exposure to predators, a common situation during transport from the TFCF, increases school cohesiveness (Krause 1993; Tien *et al.* 2004). However, school disruption, by disorientation or any other means, increases predation risk, energetic output, and stress (Rubenstein 1978). Energetic loss and stress, if significant, can be

immunosuppressive and result in decreased resistance to pathogens and reduced survival (Houghton and Matthews 1986; Pickering and Pottinger 1985, 1989). Anecdotal evidence provided by TFCF biologists suggest conditions that likely result in disorientation during transport include turbulent flows and inadequate light levels (Bridges, pers. comm.). Circular tanks with flows that allow for unidirectional flow are recommended for transporting pelagic fish like threadfin shad (Portz *et al*. 2006) because pelagic species tend to orient into current and swim in a uniform schooling pattern (Nicholson *et al*. 1992, Ross and Watten 1998). For example, Iguchi *et al*. (2002) reported obligate schooling of ayu (*Plecoglossus altivelis*) induced by whirling circular flows reduced stress during transport. Visual cues are also important in school formation (Breder 1951, Hemmings 1966, Shaw 1978). Though species specific (see Guthrie and Muntz 1993), the structure of fish eyes are similar to other vertebrates and require light to pass though the cornea and for the lens to focus light on the retina where photoreception occurs (Helfman *et al*. 1997, Moyle and Cech 2002). Therefore, the visual range, or object recognition criteria of fish, depends on light intensity (Aksnes and Giske 1993). It is plausible to assume low light levels during transport could contribute to disorientation and school disruption.

Fish transport tanks used at the TFCF are not circular in design, and provide no unidirectional flow or light during fish transportation. It is important to investigate whether light or circular flow during fish transport results in schooling of threadfin shad and if obligated schooling reduces stress, external tissues damage, and fish mortality.

Problem Statement

Fish transportation operations at the Tracy Fish Collection Facility and John E. Skinner Delta Fish Protection Facility result in the annual truck transportation of millions of threadfin shad. Given the schooling nature of threadfin shad large groups can enter the facilities over a short period of time, making the transportation of high densities of shad a necessity. Transporting fish at high densities can be stressful and lead to fish mortality, so it is necessary to investigate methods to minimize stress, external tissue damage, and mortality under such circumstances. Because schooling reduces stress in some pelagic species of fish it is necessary to investigate whether light or circular flow during fish transport results in schooling of threadfin shad and if obligated schooling reduces stress, external tissues damage, and fish mortality.

Goals and Hypotheses

Goals:

1. Determine if light affects schooling of threadfin shad during simulated transport.

2. Determine if circular flows affect schooling of threadfin shad during simulated transport.

3. Determine if obligated schooling affects threadfin shad physiological stress.

4. Determine if obligated schooling affects threadfin shad metabolic rates.

5. Determine if obligated schooling affects threadfin shad external tissue damage.

6. Determine if obligated schooling affects threadfin shad short-term survival (168 h).

Hypotheses:

1. If exposure to light induces schooling in threadfin shad, then randomly selected pairs of threadfin shad exposed to light and tracked for a 20 s period should maintain a closer proximity to eachother compared to threadfin shad exposed to minimal light conditions.

2. If exposure to circular flow induces schooling in threadfin shad, then randomly selected pairs of threadfin shad exposed to circular flow and tracked for a 20 s period should maintain a closer proximity to eachother compared to threadfin shad exposed to minimal light conditions.

3. If induced schooling in threadfin shad affects physiological stress, then schooling fish should have lower plasma cortisol, glucose, and lactate concentrations compared to threadfin shad not schooling.

4. If induced schooling in threadfin shad affects fish metabolism, then schooling fish should have lower ammonia production rates compared to threadfin shad not schooling.

5. If induced schooling in threadfin shad affects external tissue damage, then schooling fish should have lower external tissue damage compared to threadfin shad not schooling.

6. If induced schooling affects the short-term survival (168 h) of threadfin shad, then schooling fish will have lower mortality compared to threadfin shad not schooling.

Materials and Methods

Source and Care of Fish

Adult threadfin shad will be netted from schools of wild fish entrained at Reclamation's TFCF in Byron, California, truck transported to the Denver Technical Service Center (Denver, Colorado) and held in continuously aerated 757-L circular flow-through tanks at Reclamation's greenhouse. Water temperatures will be maintained at transport temperature ±0.5°C (where initial target temperature will be the temperature at which they were salvaged) and fish will be maintained under a natural photoperiod using a time controlled light system. Fish will be fed an appropriate diet at 3–4% body weight per day. When changes in water temperature are required to meet our experimental design, rate of change will be $\leq$1.0°C/d. Prior to testing fish will randomly be isolated as a function of treatment condition into eight individual holding tanks and provided a

unique mark (with eight total mark combinations) using a fluorescent microsphere solution (New West Technologies, Santa Rosa, California). This will permit the consolidation of fish during our post-treatment survival analysis and conservation of tank space. After marking, fish will be maintained at holding conditions for at least two weeks, during which mortality rates and feeding will be monitored to assure fish are healthy prior to experimentation.

Preliminary Field Data (Year 1)

The preliminary phase of this research will require us to measure current light levels during standard transport operations at the TFCF and potential light levels that may be achieved in the future if deemed appropriate. We will investigate the potential use of a window-type system as well as underwater lights to increase light levels during transport. If possible, we may use a different container with a similar shape and water capacity that we can manipulate. We will simulate the most logistically pleasing conditions during our laboratory-based experiment. In year 1, we will also work with a hydraulic engineer to see if it is feasible to create circular velocities in the TFCF fish transportation tanks using combinations of submersible pumps. A numerical model will be created in FLOW-3D Version 9.2 to map velocities in the TFCF fish transportation truck to accurately describe flow patterns (See Higgs and Demoyer 2007)

Experimental Design: Effects of Density and Water Velocity on Schooling Behavior of Threadfin Shad (Year 2)

Because of the likely challenges involved in developing and sustaining circular flows in the TFCF fish transport trucks, we deemed it necessary to measure the lowest flows possible that will induce schooling of adult threadfin shad. This measurement is necessary because exceedingly high velocities are not only unnecessary but will likely result in greater impingment of smaller fish and debris, and contribute to energetic loss as a result of swimming. We will accomplish this in year 2 (year 1 if funding is available) by inserting 50 shad (n = 8) into a 190-L tank and increasing water velocities by 2 cm/s increments every 10 minutes (min) until schooling occurs. Minimum school inducing velocities will be used to determine the effects of density on schooling behavior and in Phase 3: *Effects of light and circular flow on obligated schooling.*

Reclamation biologists have also expressed concern that when exposed to high densities, stress and disorientation may affect threadfin shad behavior, resulting in a delayed schooling response. To determine the effects of density on schooling response of shad we will be place fish into 90-L circular tanks at three densities (n = 8; 50, 100, 150 g/L), expose them to our measured minimum school inducing velocity, and record schooling behavior at 15, 30, 45, and 60 min. During both water velocity and density experiments fish schooling will be quantified as the average distance and angle, treating the fish vertebrae as a straight line, between two randomly selected neighboring individuals over a 10-s period using digitally recorded frames (see *Fish Schooling* below).

Experimental Design: Effects of Light and Circular Flow on Obligated Schooling (Year 3)

We will have four treatment conditions for our experiment: dark conditions (current transport conditions), light conditions, dark conditions + circular water velocity, light conditions + circular water velocity. A spray bar (*i.e*., waterline) oriented perpendicular to and directly against the tank wall will be used to create circular velocity in our experimental tanks. Our target water velocity will be determined during year 2 research (see above). Light and dark conditions will be achieved to simulate current conditions and potential light conditions in the TFCF fish transport truck. Bentonite clay will be added to all treatment tanks to achieve mean annual turbidity levels at the TFCF (15 NTU, Craft *et al*. 2008). To mimic the seasonal range of temperatures threadfin shad are collected and transported at the TFCF, we will conduct our experiments at 8, 16, and 24°C (Craft *et al*. 2008). Our simulated fish transport system will consist of eight 95-L tanks (filled to 76-L) and a 1600 HP/8000 Watt gas powered generator (Briggs and Stratton PRO8000) situated on a flatbed trailer. Above each individual tank we will suspend a digital video camera to record fish movements. Black sheets of plastic will be used to create appropriate light level conditions for our dark condition and dark condition + circular velocity treatments.

One-hundred fish will be provided a distinguishable mark and inserted into one of thirty-two 95-L holding tanks (8 tanks for each treatment) at a density of approximately 10 g/L, then allowed 7 d to recover from handling and marking. We will attempt to complete eight replicates per treatment per day, but will sacrifice randomization between treatments to minimize time and experimental cost. For each replicate, two control fish will be removed using water to water bucket transfer prior to treatment exposure for plasma and fluorescein analyses, respectively. After removal of control fish eight tanks, designated for one treatment, will be drained to 20-L and poured into each of the eight tanks on our simulated fish-transport system. This process is intended to simulate the fish loading process that occurs at the TFCF. Once fish have been inserted into the fish-transport system, the selected treatment will be enacted, all cameras will begin recording, and the generator will be turned on (to simulate vibrations that occur during transport). Every 5 min throughout the 60-min test period the transport tanks and trailer will be manually moved forward (or backwards) to create water sloshing in the tanks, a process that occurs upon each stop or turn while transporting fish from the TFCF. After simulated transport two fish will be removed using water to water bucket transfer for plasma and flourescein analyses, respectively, and 20 fish from each tank will be removed and transferred to one common 190-L tank for 168-h survival assessment. All remaining fish will be measured for length (fork, standard, and total lengths in mm) and wet weight (g). Water quality (ºC, pH, and TAN) and survival will be monitored daily through 168 h.

Plasma Analysis

Blood samples will be immediately centrifuged for 4 min at 12,000 × g, effectively separating blood plasma from packed cells. Blood hematocrit levels for each individual sample will be recorded immediately, and the plasma will be transferred to cryogenic freezing vials and stored in a liquid-nitrogen dewar flask. Plasma lactate and glucose concentrations will be measured with a polarographic analyzer (YSI 2700 Select,

Yellow Springs, Inc., Yellow Springs, Ohio), and plasma cortisol concentrations will be measured by the University of California Davis Endocrinology Laboratory using a modified enzyme immunoassay.

External Tissue Damage

Scale loss and external tissue damage will be determined in the control and the two treatment groups immediately post-treatment using fluorescein (AK-Fluor®, Akorn, Inc., Decatur, Illinois). Fluorescein is a nontoxic fluorescent dye that can be used to rapidly and easily detect scale loss and tissue lesions and ulcers by binding to breaks or tears in the epithelial barrier of soft tissue. Fish will be anesthetized in a MS-222 bath (40 mg/L) and transferred to a solution of 0.20-mg fluorescein/1ml water for 5 min and then rinsed in three separate clean water baths for 2 min. The fish will then be euthanized in a 200 mg/L MS-222 bath and immediately examined for skin damage under an ultraviolet light (Model UVGL-58, Mineralight, Upland, California). Photographs are taken in complete darkness under ultraviolet light using a Nikon D-100 digital camera. Severity of tissue damage will be categorized, external bacterial infections will be diagnosed, and total damaged area will be quantified. Weights (±0.01 g) and measurements (TL, ±1 mm) of each fish using an electronic balance and fish measuring board will be recorded.

Fish Schooling

Fish schooling will be quantified as the average distance and angle, treating the fish vertebrae as a straight line, between two randomly selected neighboring individuals over a 10-s period using digitally recorded frames. Ten second blocks from each digital video camera will be analyzed at 15, 30 and 45 min after the initiation of each group of replicates. The closer two individual fish are together and the closer to parallel of each representative line the more likely the two fish were in a schooling pattern.

168-h Mortality Analysis

After simulated transport fish will be monitored daily. Dead fish will be removed, identified by mark, and measured for length (fork, standard, and total lengths in mm) and wet weight (g). After 168 h is complete all remaining fish from each treatment will be measured for length and weight.

Field Verification (Year 4)

If our laboratory data proves light and/or circular water velocity during simulated fish transport improves stress, damage, and/or survival, we will validate these data using a larger volume transport system (1100-L on TFCF F450 truck) and water from the SSJD.

Sample Size, Fish Needs and Estimate of Time Required for Completion

A power calculation was carried out using post transport fish plasma constituent data from Iguchi *et al*. 2002, whom assessed the effects of whirling water (circular flow) and turbulent water on cortisol levels of ayu (*Plecoglossus altivelis*). Based on mean cortisol concentrations of ayu 24 h after exposure to transport in whirling and turbulent waters we wanted to be able to detect a difference of 20 ng/mL using their reported

standard deviation. Our desired power level is 0.90 and our alpha level is 0.05. We used SAS version 9.1, a statistical software package published by the SAS Institute, Inc., to run the power calculation. Based on this calculation the minimum sample size needed to provide the desired power level, where sample size per group = n/2, is 12. However, given our experimental set-up, the elevated variance observed when assessing fish survival and external damage in our current research, and the minimal cost incurred to conduct additional replicates, we will target a sample size of n=16.

If we use 100 threadfin shad pre treatment, employ four treatment conditions and three test temperatures, we will need to mark 19,200 fish. Assuming 10% mortality we will require 21,120 threadfin shad for testing. Assuming we can complete eight replicates per treatment each day, it requires 2 d for two technicians to mark fish (at a rate of 1600 fish/d) for the subsequent test day, and we allow 7 d for acclimation post-marking, it will take us approximately 22 d to complete 16 replicates. Assuming we employ three test temperatures we will require approximately 86 d for testing.

Data Analysis

If assumptions necessary to model using parametric statistics (normality and equality of variance) are achieved, a two-way analysis of variance (ANOVA) and Tukey's test for multiple comparisons will be used to test for differences between plasma constituent levels (hematocrit, lactate, glucose and cortisol), damage, schooling, and 168-h mortality levels for controls and treatment × temperature combinations. If ANOVA assumptions are not met, Kruskal-Wallis ANOVA on ranks and Dunn's test will be employed. All statistical analyses will be conducted using Sigmastat 3.0 (Jandel Scientific, San Rafael, California) statistical software with an alpha level for all analyses set at 0.05.

Coordination and Collaboration

Experimental design and research updates will be provided at requested TTAT and/or CVFFRT meetings. However, primary coordination and collaboration will be between TFCF staff and biologists, the Fisheries and Wildlife Resources Group, SAIC government contractors, and the interagency TTAT.

Endangered Species Concerns

Fish species used in this study, threadfin shad and striped bass, are non-native to the SSJD and are not listed under the Federal or California State Endangered Species Acts (ESA). Both species will be collected at the TFCF under permitted quantities by Reclamation employees. California Scientific Collecting Permit 802025-02 has been obtained for all primary investigators and all test species.

Dissemination of Results

Research updates will be provided and/or presented at regularly scheduled Tracy Technical Advisory Team (TTAT) and Central Valley Fish Facilities Review Team (CVFFRT) meetings. The primary deliverables will be a Tracy Volume Series, as well as a publication in a peer-reviewed scientific journal. However, posters and/or oral presentations will also be given at appropriate scientific meetings (*i.e.*, American Fisheries Society). Additionally, information obtained in this study will be used in the

implementation of new fish transportation tables for use at south SSJD fish collection facilities.

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